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Prevalence and Effects of *Theileria Spp*. on Hematological Parameters in Dromedary Camels (*Camelus dromedarius*) in Northeastern Libya

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ABSTRACT

Theileriosis is a hemoprotozoan infection spread by ticks and caused by Theileria spp., representing a serious health concern for dromedary camels (Camelus dromedarius) across Africa and the Middle East. However, its prevalence and hematological impacts in northeastern Libya remain poorly understood. This study aimed to determine the prevalence of *Theileria spp.* infection in dromedary camels in northeastern Libya also to evaluate its effects on hematological parameters. Blood samples were collected randomly from 160 dromedary camels across diffrent localities in northeastern Libya between February 2021 and January 2022. Diagnosis was performed using microscopic examination of Giemsa-stained thin blood smears. Hematological analysis was conducted using an automated hematology analyzer (MEK-6510K, Nihon Kohden, Japan). Statistical differences between infected and non-infected were evaluated using t-test and ANOVA. The overall prevalence of Theileria spp. infection was 85% (136/160). Parasites appeared within erythrocytes in ring, comma, oval forms, and schizonts were observed as blue bodies within lymphocytes. Infected camels exhibited macrocytic anemia characterized by a significant decrease in red blood cell count (RBC: 4.83 ± 0.21 × 10^6/µL) and hemoglobin concentration (Hb: 10.58 ± 0.22 g/dL), alongside a marked increase in mean corpuscular volume (MCV: 78.01 ± 5.34 fL). Leukocytosis was also observed (WBC: 17.95 ± 0.99 × 10^3/µL), driven primarily by neutrophilia. Conclusions: Theileriosis is highly endemic in northeastern Libya and significantly impairs the health status of dromedary camels. Urgent interventions including tick control programs and routine hematological monitoring are recommended. Molecular identification of circulating Theileria species is warranted in future studies.

1. INTRODUCTION

The dromedary camel (*Camelus dromedarius*) plays important role in the economies and food security of arid and semi-arid regions of Africa and Asia. Often called (the ship of the desert) the dromedary provides meat, milk, transport, and wool to millions of people who depend on it for their livelihoods. The global camel population was estimated at approximately 35–38 million animals, with the vast majority around 80% located in Africa and the Middle East (Faye, 2020; Sazmand *et al*., 2019). In Libya, camels are deeply embedded in cultural identity and serve as important assets for rural and Bedouin communities, particularly in the northeastern and southern regions of the country.Despite their well-known physiological resilience to extreme heat, dehydration, and sparse nutrition, dromedary camels are susceptible to a wide range of infectious diseases, especially tick-borne parasitoses. Among the most clinically significant are hemoprotozoan parasites of the genus *Theileria*, which are obligate intracellular protozoa transmitted primarily by hard ticks *(family Ixodidae*), especially those of the genus *Hyalomma* (Selim *et al*., 2023). Theileriosis in camels manifests clinically as fever, lethargy, weight loss, anemia, lymphadenopathy, and, in severe cases, death, leading to significant economic losses in the livestock sector. The life cycle of *Theileria* involves two distinct phases: a sexual phase in the tick vector and an asexual phase in the mammalian host. In the vertebrate host, sporozoites injected during tick feeding first invade lymphocytes where they undergo schizogony (forming schizonts), followed by invasion of erythrocytes as piroplasms (merozoites). It is this erythrocytic stage that causes hemolysis, leading to anemia and the characteristic hematological disturbances associated with theileriosis (Selim *et al*., 2023; El-*Alfy et al*., 2024). Several studies have reported the prevalence of *Theileria* spp. in dromedary camels across different countries. Ismael *et al.* (2014) documented an outbreak of theileriosis in Saudi Arabia with significant hematological alterations, while Youssef *et al*. (2015) reported macrocytic hypochromic anemia associated with *T. annulata* infection in camels in Egypt, with prevalence rates ranging between 44.8% and 71.9%. Al-Malki and Hussien (2022) reported a prevalence of 38.73% in Saudi Arabia. In Libya, however, data on this subject remain extremely limited; the only previous study from western Libya reported a prevalence as low as 6.5% (EL-Maghrbi and Hosni, 2008), which stands in sharp contrast to the situation in the northeastern regions. Hematological parameters are widely recognized as reliable indicators of the physiological and pathological status of animals. Monitoring hematological parameters such as red blood cell (RBC) indices, hemoglobin (Hb) levels, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and the differential count of white blood cells (WBCs) is important for evaluating the intensity of infection and assessing the overall physiological status of the host (Osman et al., 2015; Mahran, 2004). Given the scarcity of data on theileriosis in northeastern Libya, this study was designed with two primary objectives: (1) to determine the prevalence of *Theileria* spp. infection in dromedary camels in this region using microscopic examination of Giemsa-stained blood smears, and (2) to evaluate the impact of this infection on hematological parameters. The findings of this study will contribute to a better understanding of the prevalence of camel theileriosis in Libya and provide a scientific basis for developing effective disease control strategies.

2. METHOD

2.1 Study Area and Study Design

This cross-sectional study was conducted in northeastern Libya between February 2021 and January 2022. The study area encompasses ten different localities across the region, which is characterized by a Mediterranean climate with hot and dry summers and mild winters conditions that are highly favorable for tick proliferation and survival. Camels in this area are typically managed under extensive or semi-extensive pastoral systems with limited veterinary supervision, which increases their exposure to ectoparasites, including *ixodid* ticks.

2.2 Animal Selection and Sample Collection

A total of 160 camels (*Camelus dromedarius*) were randomly selected from the different localities. All sampled animals were subjected to a brief clinical examination prior to blood collection. Venous blood samples (5 mL each) were collected aseptically from the jugular vein using sterile disposable syringes fitted with 19–20-gauge needles. Each sample was transferred immediately into sterile vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent clotting. All tubes were properly labeled and transported on ice to the laboratory at the College of Veterinary Medicine, Omar Almukhtar University, Al-Bayda, Libya, for analysis.

2.3 Microscopic Examination

Thin blood smears were prepared from each EDTA blood sample immediately after collection. Smears were allowed to air-dry at room temperature, then fixed with absolute methyl alcohol (99.5%) for one minute. The fixed smears were subsequently stained with 10% Giemsa stain for 25 minutes and rinsed gently with distilled water. The dried slides were examined under a light microscope (Olympus CX23, Japan) using an oil immersion objective at 100× magnification. Each slide was examined systematically across at least 100 fields to detect the presence of *Theileria* piroplasms within erythrocytes and schizonts within lymphocytes. Infection was confirmed by the characteristic morphology of the parasite, including ring, comma, and oval forms inside red blood cells.

2.4 Hematological Analysis

Whole blood samples were analyzed using an automated veterinary hematology analyzer (MEK-6510K, Nihon Kohden, Japan) to determine the following parameters: total red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and total white blood cell count (WBC). Differential leukocyte counts—including percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils—were performed with Wright-stained blood smears under the same microscope.

2.5 Statistical Analysis

The prevalence of theileriosis was calculated as the number of infected camels divided by the total number examined, expressed as a percentage. Data were expressed as mean ± standard error of the mean (Mean ± SE). Statistical comparisons between the hematological values of infected and non-infected camels were performed using the independent-samples t-test. One-way analysis of variance (ANOVA) was used for comparisons across multiple groups where applicable. All statistical analyses were performed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). At p-value of < 0.05 was considered statistically significant.

3. ETHIC APPROVAL

Al-Mukhtar committee for Biosafety and Bioethics (MCBB) has reviewed and discussed the application to conduct the above-mentioned research in Oumar Al-Mukhtar University.
The documents have been received, reviewed and approved by the Al-Mukhtar committee chairman, and was given this reference number: **NBC: 007. A. 26. 82.**
The approved research protocol, to be conducted in the presented form and confirm that neither the investigator nor the co-investigator(s), participating in this research project were involved in voting and decision making. The MCBB, expects to be informed about**:**
1. The progress of the research project.
2. Any modifications occurring in the research protocol.
The MCBB is working according to its own guidelines and international applicable guidelines.

4. RESULT

4.1 Prevalence of *Theileria spp*. and Morphological Characteristics

The total of 160 dromedary camels examined 136 were found positive for *Theileria* spp. infection by microscopic examination of Giemsa-stained thin blood smears, yielding an overall prevalence of 85% (136/160) as fig (1).

Parasites were observed morphologically within erythrocytes as small intraerythrocytic piroplasms appearing in three distinct forms: ring-shaped, comma-shaped ("Maltese cross" or comma), and oval-shaped forms. The oval form was particularly characteristic and abundant in positive samples in fig. (2).

.In addition to erythrocytic stages, schizonts were identified as blue bodies (Koch's blue bodies) within lymphocytes, confirming active lymphocytic invasion by the parasite. No co-infections with other blood parasites (e.g., *Babesia* or *Trypanosoma* spp.) were confirmed in the examined smears.in fig. (3)

4.2 Hematological Parameters in Infected Camels

Hematological analysis revealed significant alterations in infected camels compared to the healthy dromedary camels. The findings are summarized in Table (1).

Infected camels showed a significant decrease in RBC count ($4.83 \pm 0.21 \times 10^6/\mu L$) compared to the lower limit of the healthy camels ($7.5 \times 10^6/\mu L$), representing a reduction of approximately 36%. Similarly, Hb concentration was significantly reduced (10.58 ± 0.22 g/dL; $p < 0.05$). However, PCV values ($28.99 \pm 1.14\%$) remained within the normal range (25–35%), suggesting a compensatory mechanism. A striking finding was the marked elevation in MCV (78.01 ± 5.34 fL), which was far above the healthy camels (27.0–33.0 fL, indicating macrocytic erythrocytes. MCHC values (45.00 ± 1.74 g/dL) remained within the normal range (42.0–49.6 g/dL).

Total WBC count was significantly elevated in infected camels ($17.95 \pm 0.99 \times 10^3/\mu L$), exceeding the upper normal limit of $16.0 \times 10^3/\mu L$, indicating leukocytosis. Differential leukocyte counts revealed neutrophilia ($51.95 \pm 1.23\%$) as the predominant component of the leukocyte response, alongside a lymphocyte percentage of $40.30 \pm 1.23\%$, which was within the upper boundary of the healthy camels

5. DISCUSSION

Prevalence and Epidemiological Significance The current study recorded a remarkably high overall prevalence of *Theileria* spp. in dromedary camels in northeastern Libya, with 85% (136/160) of examined animals testing positive by microscopic examination. This is, to our knowledge, one of the highest prevalence rates ever reported for camel theileriosis in the region. This markedly exceeds prevalence rates reported in many neighboring and comparable countries. For instance, Al-Malki and Hussien (2022) reported a prevalence of 38.73% in Saudi Arabia, while El-Alfy *et al.* (2024), varying prevalence rates reported across North Africa and the Arabian Peninsula. Studies from Egypt reported rates ranging between 44.8% and 71.9% (Youssef *et al*., 2015), while the only previous Libyan study, conducted in the western part of the country, reported a prevalence of only 6.5% (EL-Maghrbi and Hosni, 2008). The vast difference between our results and those from western Libya likely reflects distinct ecological and epidemiological conditions between the two regions.

The exceptionally high prevalence observed in northeastern Libya can be attributed to several interacting factors. First, the northeastern coastal and inland zones of Libya are ecologically suitable for the survival and proliferation *of ixodid* tick vectors, particularly *Hyalomma dromedarii* and *H. anatolicum*, which are the primary vectors of *Theileria* spp. in camels (Selim *et al*., 2023). The warm, semi-arid climate with mild winters provides year-round conditions favorable for tick activity and population growth. Second, the extensive pastoral management practices prevalent in northeastern Libya result in camels grazing over large areas with minimal veterinary supervision, significantly increasing their contact with tick-infested environments and reducing the likelihood of timely acaricide treatments. Third, the lack of awareness among herders about theileriosis and the absence of organized disease surveillance programs contribute to the perpetuation and spread of infection within and between herds.

Another contributing factor may be the high tick burden typically observed in this region. *Hyalomma* ticks thrive in hot, dry environments and are known to parasitize camels preferentially, with high infestation rates documented throughout North Africa and the Middle East (Selim *et al*., 2023; El-Alfy *et al*., 2024). The high tick burden directly translates to increased opportunity for parasite transmission, which is consistent with the elevated prevalence recorded in this study. Furthermore, subclinical or latent infections that go unrecognized in apparently healthy animals may also inflate the detected prevalence when sensitive diagnostic techniques even microscopy are applied systematically to a large sample.

Parasite Morphology. Microscopically, the parasites observed in this study appeared within erythrocytes in three distinct morphological forms: ring-shaped, comma-shaped, and oval-shaped. These forms are consistent with intraerythrocytic piroplasms of the genus *Theileria*. The presence of schizonts (Koch's blue bodies) within lymphocytes further supports the diagnosis and confirms active parasitism at the lymphoid stage. These morphological features are broadly consistent with descriptions of *Theileria annulata* infection in camels and cattle across the region (Youssef *et al*., 2015; Ismael *et al*., 2014). However, it should be acknowledged that microscopic examination alone cannot reliably differentiate between the various species of *Theileria* that may infect camels, including *T. annulata*, *T. camelensis*, and *T. dromedarii*. Molecular tools such as PCR essential for definitive species identification (El-Alfy *et al.,* 2024).

Erythrocytic Alterations and Anemia. One of the most clinically important findings of this study was the development of macrocytic anemia in infected camels. The RBC count was significantly reduced to 4.83 ± 0.21 × 10^6/μL and Hb concentration was significantly lower than healthy camels (10.58 ± 0.22 g/dL, 10.2–16.0 g/dL) respectliy. These reductions are hallmarks of hemolytic anemia induced by erythrocyte parasitism. The parasites destroy erythrocytes both directly through intracellular multiplication and indirectly through the activation of the host's immune system and reticuloendothelial system, which removes parasitized and non-parasitized erythrocytes from circulation (Selim *et al*., 2023; Ismael *et al*., 2014). Interestingly, PCV values remained within the normal range (28.99 ± 1.14%; reference: 25–35%), which at first glance appears contradictory to the low RBC count. This discrepancy is best explained by the markedly elevated MCV (78.01 ± 5.34 fL and healthy of 27.0–33.0 fL).

The increased MCV indicates that the remaining circulating erythrocytes are significantly larger than normal a characteristic of macrocytic erythrocytes. This macrocytosis is a classic feature of a regenerative bone marrow response, where immature, larger erythrocytes (reticulocytes) are released prematurely into the bloodstream to compensate for the ongoing erythrocyte destruction. This regenerative response has been described in camels and other livestock with theileriosis (Youssef et al., 2015; Mahran, 2004) and indicates that the anemia, although significant, has not yet overwhelmed the bone marrow's compensatory capacity. The maintenance of normal PCV despite low RBC counts is a well-recognized phenomenon in macrocytic anemia, as the larger cell size partially compensates for the reduced cell number in terms of total blood volume occupied.Normal MCHC values (45.00 ± 1.74 g/dL; reference: 42.0–49.6 g/dL) indicate that the hemoglobin concentration within each individual erythrocyte is normal consistent with normochromic macrocytic anemia. Similar erythrocytic changes, including reduced RBC and Hb with elevated MCV, have been reported in camels with theileriosis in Egypt (Youssef *et al*., 2015), Saudi Arabia (Ismael *et al*., 2014), and Pakistan (Aslam *et al.,* 2023), suggesting that macrocytic normochromic anemia is a consistent pathological feature of camel theileriosis across different geographical regions. The anemia arising from theileriosis can seriously compromise the productive performance of camels, resulting in reduced milk and meat production, impaired reproductive efficiency, (Faye, 2020; Selim *et al*., 2023).

Leukocytosis and Neutrophilia. Total WBC count was significantly elevated in infected camels (17.95 ± 0.99 × 10^3/μL), well above the upper limit of the healthy (7.0–16.0 × 10^3/μL), indicating leukocytosis. Differential leukocyte analysis revealed neutrophilia as the primary driver of this leukocytosis, with neutrophil percentages of 51.95 ± 1.23% elevated above the healthy camels of 25–50%. This finding is consistent with reported from previous studies of camel theileriosis in Saudi Arabia (Ismael *et al*., 2014), Egypt (Youssef *et al*., 2015), and other countries (Azeem *et al*., 2019; Osman *et al*., 2015).

The neutrophilia observed in current study can be interpreted as a reflection of the acute phase inflammatory response triggered by the parasite. *Theileria* parasites and their metabolic by products stimulate the release of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which in turn promote neutrophil mobilization from bone marrow stores and peripheral blood marginalization. This systemic inflammatory response serves as a first-line defense mechanism against the invading parasites but can also contribute to tissue damage and pathological changes in various organs (Selim *et al*., 2023). Neutrophilia in the context of parasitic infection has also been linked to the presence of secondary bacterial infections that may develop in immunocompromised animals, a complication commonly observed in severely parasitized livestock.

Lymphocyte percentages (40.30 ± 1.23%) remained within the upper boundary of the normal range, reflecting the active stimulation of lymphoid tissues by parasitic antigens. Lymphocytes play a central role in adaptive immunity against intracellular protozoa and their sustained presence in circulation indicates that an active cellular immune response was ongoing. Previous studies have documented lymphocytosis in camels and other animals infected with *Theileria*, attributing it to the direct stimulation and proliferation of lymphocytes by schizonts within lymph node cells (Youssef *et al*., 2015; Osman *et al*., 2015). The slight elevation of lymphocytes in this study, though within the normal range, may indicate the beginning of an adaptive immune response that, in more chronic or severe infections, could develop into frank lymphocytosis.

The combined hematological disturbances documented in this study namely macrocytic normochromic anemia and neutrophilic leukocytosis reflect a significant compromise in the physiological homeostasis of infected camels. The reduction in oxygen-carrying capacity due to anemia, coupled with the energy demands of mounting an immune response, renders infected camels less productive and more susceptible to secondary infections.

These effects have direct economic consequences for camel-dependent communities in Libya, where camels are an important source of meat, milk, and draft power (Faye, 2020). Given the exceptionally high prevalence of 85% documented in this study, theileriosis must be considered a major endemic disease constraint on camel productivity in northeastern Libya, necessitating urgent attention from veterinary authorities and policymakers.

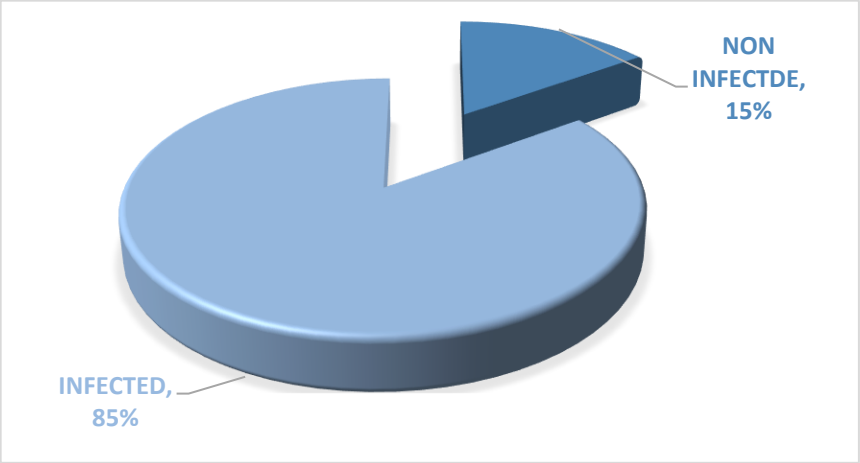

Fig. (1): The overall prevalence of Theileria. Spp. infection in Dromedary Camels (Camelus dromedarius) in Northeastern Libya.

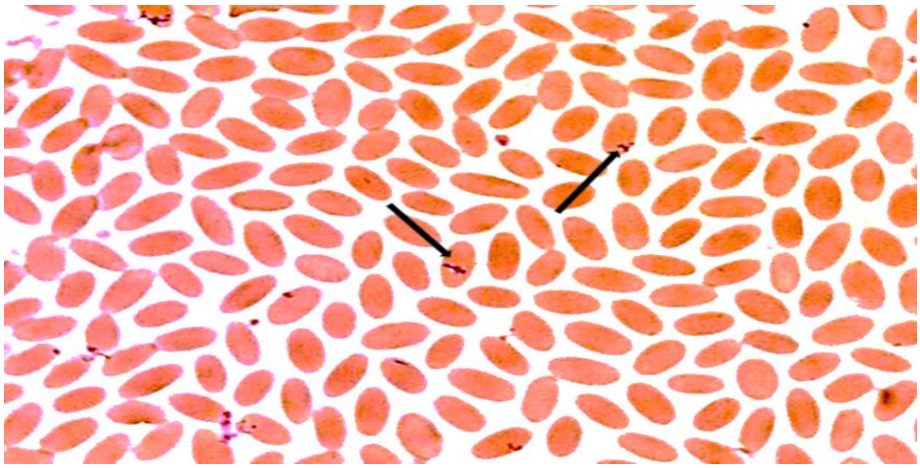

Fig (2) Blood smear of dromedary camel with Theileria spp blood parasites appeared stained with Giemsa in the infected red blood cells.

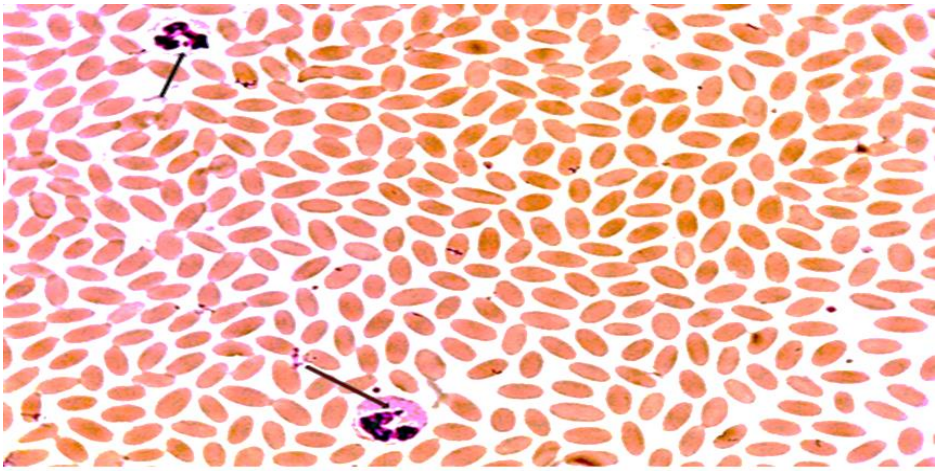

Fig (3) The parasite appears in the white-blood-cells as the Schizonts (Koch's Blue Bodies) in Lymphocytes Theileria

Table 1. Hematological parameters of Theileria spp.-infected dromedary camels in northeastern Libya compared to the healthy dromedary camels

Parameter	Infected Camels (Mean ± SE)	(Healthy Camels) (Mean ± SE)	Statistical Interpretation
RBC ($\times 10^6/\mu L$)	4.83 ± 0.21	7.5 – 12.0	Significant decrease ($p < 0.05$)
Hb (g/dL)	10.58 ± 0.22	10.2 – 16.0	Significant decrease ($p < 0.05$)
PCV (%)	28.99 ± 1.14	25.0 – 35.0	Within normal range
MCV (fL)	78.01 ± 5.34	27.0 – 33.0	Significant increase ($p < 0.05$)
MCHC (g/dL)	45.00 ± 1.74	42.0 – 49.6	Within normal range
WBC ($\times 10^3/\mu L$)	17.95 ± 0.99	7.0 – 16.0	Significant increase ($p < 0.05$)
Neutrophils (%)	51.95 ± 1.23	25.0 – 50.0	Elevated (neutrophilia)

6. CONCLUSION

This study provides the first prevelance epidemiological and hematological assessment of *Theileria* spp. infection in dromedary camels in northeastern Libya. The findings revealed high prevalence of 85%, far exceeding rates reported in other regions, and demonstrate significant hematological consequences, including macrocytic normochromic anemia and neutrophilic leukocytosis. These disturbances reflect considerable physiological stress in infected animals and have important implications for camel health, productivity, and the livelihoods of dependent communities. The results underscore the urgent need for: (1) systematic tick control programs targeting *Hyalomma* vectors across northeastern Libya; (2) regular hematological monitoring of camel herds to enable early detection of infection; and (3) future molecular studies to identify the specific species of *Theileria* circulating in the region and to better characterize their virulence and transmission dynamics. These efforts will be critical for reducing the burden of theileriosis on camel populations and improving the sustainability of camel husbandry in Libya.

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Theileria spp; Camelus Dromedarius; Prevalence; Hematology; Anemia; Tick-Borne Disease**;** Libya